



## COLONIZATION OF HAWAIIAN ACACIA KOA SEEDLINGS WITH *FUSARIUM* SPECIES

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### ABSTRACT

*Acacia koa* seedlings were evaluated from plantings and commercial nurseries for colonization by *Fusarium* spp., particularly *F. oxysporum*, the putative cause of koa wilt/dieback disease in Hawaii. We sampled 162 seedlings, 90% of which were from nurseries on the islands of Hawaii, Maui, Kauai, and Oahu. *Fusarium oxysporum* was detected on about 78% of sampled seedlings. It was most prevalent on roots; more than 50% of all sampled root pieces were colonized by this species. Ten additional *Fusarium* species were isolated from seedlings, but all colonized plant tissues at much lower levels. More than 70% of all samples from seedlings yielded *Fusarium* spp. The potential importance of this high level of colonization is discussed.

### INTRODUCTION

One of the most important tree species in Hawaii is *Acacia koa* A. Gray, which is a fast growing native (Dudley 2002). There is much recent interest in increasing koa plantings because of its high value and the availability of previous agriculture land for potential plantations. One of the major limiting factors in increased production of koa is an important wilt/dieback

disease that seems to be increasing in importance. The primary cause of this disease is believed to be the soil-borne vascular wilt pathogen *Fusarium oxysporum* f.sp. *koae* (Anderson and others 2002; Gardner 1980), although weather and site factors undoubtedly play important roles in disease severity (Anderson and others 2002).

Disease symptoms can occur on plants of all ages from young seedlings to large, old-growth

trees (Anderson and others 2002; Gardner 1980; James and others 2007). Seedlings tend to wilt and die more rapidly than older trees (James 2004a). Previous work (Gardner 1980) indicated that *F. oxysporum* may contaminate and be disseminated on koa seeds. Another major concern is the possibility of the pathogen spreading on infected nursery seedling stock. This could potentially spread the pathogen to new areas. Therefore, we conducted a survey to evaluate the extent and distribution of *F. oxysporum* and other *Fusarium* species on some recently-planted seedlings as well as on koa seedling nursery stock.

## MATERIALS AND METHODS

Planted koa seedlings exhibiting wilt symptoms were occasionally collected during field examinations of diseased areas. Seedlings grown in 20 commercial nurseries on the four major Hawaiian Islands (Hawaii, Maui, Kauai, Oahu)(Table 1) were also examined for any indication of disease or reduced vigor, i.e., wilting, foliar chlorosis or necrosis, and dieback. We tried to sample the poorest looking nursery seedlings such as those not growing well or with some foliar discoloration. However, in many cases most of our sampled seedlings appeared healthy. The number of seedlings sampled varied among the nurseries. Ninety percent of all sampled seedlings came from commercial nurseries. More than half of all sampled seedlings were from the Big Island (Table 1).

Selected seedlings were extracted from soil or their containers, kept refrigerated, their roots thoroughly washed to remove soil or growing media, and shipped to the laboratory for analysis. Roots of all seedlings were sampled for *Fusarium* colonization; stems and/or branches were sampled from only about 38% of the seedlings. Roots and stem/branch sections were dissected into pieces about 5 mm in length. Randomly-selected pieces were surface sterilized in 0.525% aqueous sodium hypochlorite (10% bleach solution), rinsed in sterile water, and placed on a selective agar medium for *Fusarium*

and closely-related fungi (Komada 1975). Plates were incubated under diurnal cycles of cool, fluorescent light at about 24°C for 7-14 days. Selected emerging fungi were transferred to potato dextrose and carnation leaf agar (Fisher and others 1982) for identification using the taxonomy of Nelson and others 1983. Percentages of sampled pieces colonized by particular *Fusarium* species were calculated.

## RESULTS AND DISCUSSION

Eleven different *Fusarium* species were isolated from seedlings (Table 2). The most commonly-isolated species by far was *F. oxysporum*; it was found on more than half of the sampled root pieces and almost a fourth of the stem or branch pieces. This species was isolated from about 78% of all sampled seedlings; it was prevalent on seedlings both in plantings and within nurseries. We found *F. oxysporum* commonly on the roots of many seedlings that displayed little or no disease symptoms. All sampled roots appeared healthy with no indications of necrosis or discoloration. Vascular discoloration was noted within stems of some, but not all, seedlings from which *F. oxysporum* was isolated.

Although *F. oxysporum* was common on most sampled seedlings, we do not know the capability of such isolates to elicit disease on koa. We plan to evaluate pathogenic potential of some of these isolates in future controlled greenhouse inoculation tests. However, at the present time we don't know what proportions of the *F. oxysporum* population colonizing seedlings are potentially pathogenic. It is possible that all or most of the isolates are either saprophytic or non-pathogenic endophytes (Gordon and Martyn 1997; Kistler 1997). Since standard wilt symptoms were not usually found in sampled nurseries, we suspect that the majority of the isolates we obtained are non pathogens. On the other hand, the planted seedlings we sampled exhibited wilt or dieback symptoms and we would expect a greater proportion the fungal population from these seedlings to be pathogenic.

We have found *F. oxysporum* commonly colonizing roots of large koa trees (James and others 2007). Roots of large trees also appear healthy, even though they may be extensively colonized. Isolates of *F. oxysporum* capable of inducing wilt symptoms initially infect cortical root cells and rapidly grow into vascular systems, where spores may be systemically distributed throughout infected plants (James 2004b; James and Dumroese 2007; Nelson and others 1981). We found that *F. oxysporum* was systemically distributed in those seedlings in which roots, stems and branches were sampled.

We are unsure about potential inoculum sources of *F. oxysporum* in koa nurseries. High levels of this species have not been routinely detected on sampled koa seeds (James 2004a; James and others 2006). Plastic and styrofoam containers reused for several seedling crops may be important sources of pathogen inoculum (James 2004b; James and Dumroese 2007). Most of the containers used in Hawaiian nurseries were plastic tubes of varying sizes. Previous work (Dumroese and others 1993, 2002) indicated that *Fusarium* inoculum can remain viable on such containers for prolonged periods; sterilization with hot water is usually required to remove inoculum. Another potential source of *F. oxysporum* inoculum is the growing media used by nursery growers, although commercial peat-based media is usually not contaminated by potentially-pathogenic *Fusarium* (James 1985). We have not tested the media and containers used by Hawaiian nursery growers for potential pathogens. Some media contained rather large pieces of wood and volcanic rock and it is possible that this material may potentially harbor *Fusarium* inoculum.

*Fusarium oxysporum* is usually soil-borne and many of the sampled nurseries are located in areas where wind-blown soil contamination occurs. Therefore, blowing soil may be an important source of inoculum within nurseries, although this has not yet been tested.

The other most common *Fusarium* species isolated from koa seedlings were *F. solani* (Mart.) Appel & Wollenw., *F. semitectum* Berk. & Rav. and *F. subglutinans* (Wollenw. & Reinking). *Fusarium solani* is a common soil-borne fungus capable of causing several types of plant diseases, including root decay (James 2004b; Nelson and others 1981); we have found it most commonly within the interior portions of large roots, stems and branches of koa trees exhibiting wilt/dieback symptoms (Daehler and Dudley 2002; James and others 2007). *Fusarium solani* is often associated with activity of black twig borers (*Xylosandrus compactus* Eichhoff (Coleoptera: Scolytidae), which commonly infest declining trees (Daehler and Dudley 2002).

Both *F. semitectum* and *F. subglutinans* are commonly isolated from *Acacia koa* seed (James 2004a; James and others 2006). These species have also been detected on larger trees with disease symptoms, although at relatively low levels (James and others 2007). We suspect that some seedlings become infected from contaminated seeds following germination. Although both *Fusarium* species may be plant pathogens (Logreico and others 2002; Onyike and Nelson 1992; Satou and others 2001), their abilities to elicit diseases on koa seedlings have not been adequately tested. Limited tests indicated that both *F. subglutinans* and *F. semitectum* have pathogenic potential on young koa seedlings under controlled greenhouse inoculation conditions (Dudley and others 2007). However, much further testing will be necessary before we can make definitive conclusions regarding the pathogenic potential of these two *Fusarium* species.

The other seven *Fusarium* species isolated from koa seedlings were found at very low levels (Table 2). We suspect that few, if any, of these are capable of causing koa diseases. Most of them may be non-pathogenic endophytes that do not adversely affect their hosts.

In conclusion, we confirmed that *F. oxysporum* commonly colonizes *Acacia koa* seedlings from

both plantings and commercial nurseries. However, nursery growers need not be concerned unless the majority of isolates within their nurseries are pathogenic. Determining pathogenic potential of *F. oxysporum* populations usually requires expensive, time-consuming inoculation tests. However, in some cases specific molecular genetic markers have been developed to differentiate pathogenic from non-pathogenic isolates (Bao and others 2002; Lori and others 2004; Roncero and others 2003). With such markers, characterization of fungal populations may be determined much more quickly and at less cost. Using such techniques to characterize *Fusarium* populations associated with koa seedlings will greatly improve our understanding of the potential impacts of these fungi on nursery crops.

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Table 1. Locations where *Acacia koa* seedlings were sampled for colonization by *Fusarium* spp.

Island [County]	Number of Nurseries Sampled <sup>1</sup>	Percent of Seedlings Sampled
Hawaii [Big Island]	7	57
Maui	6	28
Kauai	4	12
Oahu	3	3

<sup>1</sup>90%

Table 2. Colonization of *Acacia koa* Seedlings with *Fusarium* spp.

Number of Seedlings Sampled	Roots	Stems/Branches	All Samples
	158	62	162
Pieces Sampled	1892	533	2425
<i>Fusarium</i> spp.	Percent Colonization		
<i>oxysporum</i> <sup>1</sup>	56.1	21.2	48.4
<i>solani</i>	9.6	8.1	9.2
<i>semitectum</i>	7.9	6.0	7.5
<i>subglutinans</i>	4.3	4.1	4.3
<i>equiseti</i>	1.4	3.6	1.9
<i>proliferatum</i>	1.2	0.9	1.1
<i>avenaceum</i>	0.05	3.6	0.8
<i>graminearum</i>	0.8	0.2	0.7
<i>acuminatum</i>	0.4	0.4	0.4
<i>sporotrichioides</i>	0.4	0.2	0.4
<i>anthophilum</i>	0	0.9	0.2
All <i>Fusarium</i>	77.1	46.1	70.3
No Fungi	0.2	36.0	8.1

<sup>1</sup>77.8% of all sampled seedlings were infected by *F. oxysporum*.

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